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APPLICATIONS OF HYBRID ULTRASONIC-ELECTROSTATIC LEVITATION TO CRYSTAL GROWTH AND DROP DYNAMICS

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ABSTRACT

The synergistic capabilities of ultrasonic and electrostatic fields have been applied to the levitation of single and compound charged and uncharged droplets in order to develop novel experimental methods for studying solution crystal growth and nonlinear drop dynamics. In the first case, a protein solution droplet bearing a surface charge is electrostatically levitated and rotated along a horizontal axis during the crystal nucleation and growth phases. Ultrasonic streaming and radiation stresses are used to induce the sample rotation and the periodic modulation of its shape. The purpose of this study is to create controlled crystal growth conditions and apparatus which would reproduce some of the aspects of the low-gravity environment. In the second case, charged or uncharged drops are levitated under the combined action of the ultrasonic and electrostatic fields, and their response to time-varying driven modulation of the resulting stresses is analyzed in the nonlinear region. In addition to simple and compound droplets, we are investigating the dynamics of levitated single thin liquid shells and foam-like aggregates. The effects of both the ultrasonic and electrostatic fields on liquid film thinning and bubble coalescence are the subjects of interest.

INTRODUCTION

The work described in the first part of this paper deals with protein crystal growth within an electrostatically levitated solution drop. The aim for this research is to simulate some of the effects of microgravity in a ground based laboratory by averaging out the effective gravitational acceleration within a solution droplet by means of periodic drop rotation along a horizontal axis. The drop rotation stays at constant rate or is altered to a specific value depending on the neucleation and the growth characteristic of the protein. The levitation and rotation capabilities of an ultrasonic-electrostatic hybrid system will be described, and the results of fluid motions internal and external to the levitated droplet by means of following tracer particles will also be examined. The actual growth of Lysozyme crystals in a temperature and humidity-controlled environmental chamber under levitation and rotation will be described. The complex motion of the crystals within the drop results in part, from a combination of the centrifugal, viscous drag, gravitational, and rotation axis orientation. Recent observations indicate that the acoustic streaming flow required for drop rotation can apply a slow internal fluid motion along the direction parallel to the rotation axis.

In the second part of the paper, we address the capabilities of the hybrid system for the experimental investigation of freely suspended charged and uncharged single drops under the influence of steady and /or time-varying ultrasonic and electric fields. The primary advantage of using electrostatic levitation in an Earth-based environment is the ability to study *spherical* droplets several millimeters in diameter. Ultrasonic levitation of drops of such size under full gravity introduces significant distortion of the equilibrium shape, and the presence of large acoustic stresses also influences the motion of the interrogated drops. The introduction of an electric field in the absence of free charges allows the control of the static drop shape and of its modulation by using a time-varying E field. The addition of free surface charges introduces the further flexibility to trade-off the effects of ultrasonic and electrostatic levitation forces, thus allowing an assessment of their respective action on the dynamics of drops. To illustrate the application of this hybrid approach, we will describe results obtained in the investigation of the large amplitude, electrically-driven shape oscillations of ultrasonically levitated uncharged drops in 1 G. Typical nonlinear effects such as sub-harmonic mode drive and hysteresis have already been uncovered, and an unexplained instability onset mechanism has been observed.

1. THE ULTRASONIC-ELECTROSTATIC HYBRID LEVITATION SYSTEM

Containerless protein crystal growth was first introduced by Rhim and Chung (1991) who used electrostatic levitation. Their technique has been further improved by combining the torque generation and control capabilities of the ultrasonic levitator. The combined hybrid system is essentially, a single-axis ultrasonic levitator (Trinh, 1990) with its ultrasonic radiator and the reflector used as ground and high voltage electrodes, respectively. The combined system is capable of functioning fully as independent ultrasonic or electrostatic levitators as well as in hybrid mode by varying the relative strength of either the ultrasonic or electric fields. When a charged drop is suspended electrostatically in a hybrid system, a low ultrasonic power may be used to generate a precisely controlled torque. The system is placed inside a chamber where the temperature and the humidity is controlled.

The hybrid system is schematically described in figure 1. A 4x4x4 inches lucite chamber which surrounds the dual purpose transducer(ground electrode) and reflector(high voltage electrode) pair is placed inside another larger lucite enclosure where a constant temperature is maintained within the range of -3° C and 50° C with the stability of 0.1° C. The temperature control inside the larger enclosure is accomplished by closed-loop mixing of cold and warm air.

For controlled evaporation or growth of suspended drop, a humidity controller is implemented in the processing chamber. The simplest but most accurate humidity control is accomplished by placing a reservoir liquid at the bottom of the levitation chamber. After equilibrium(humidity close to or at 100%) is established, a drier air supply connected to the chamber via a small aquarium pump is turned on or off depending on the desired humidity. This method is proven to be slow for reaching a desired humidity, but for protein crystal growth that takes days to weeks, it is quite adequate. For drop dynamics or other fast growing crystals other more involved methods may be applied, such as the mixing of humidified and dry air, or the use of an ultrasonic liquid-on-demand nebulizer. All these methods have been tried, and they are proven effective depending on the needs.

A 10 to 50μl solution droplet is deployed through an accurate pipetor onto a needle tip with its end bent into a shape of circle to accomodate the solution. The droplet is then inserted between the reflector and the transducer where it is charged by induction upon applying a high voltage on the reflector. The charged drop is then suspended ultrasonically as the needle rapidly retracts. Electrostatic levitation then takes over, and the ultrasonic power is allowed to be reduced to about 5 to 10% of original level. Typical levitation voltage at this stage for 4mm diameter drop is about 7.5kv and the ultrasonic sound pressure, about 135dB. The droplet is then set into rotation about a horizontal axis, and allowed to equilibrate. Nucleation and crystal growth proceed under constant drop rotation. Recently, angular speed control based on the optical sensor and FFT technique (Biswas, 1991) have been developed to control the rotation rate accurately(within 0.02 Hz) for a long

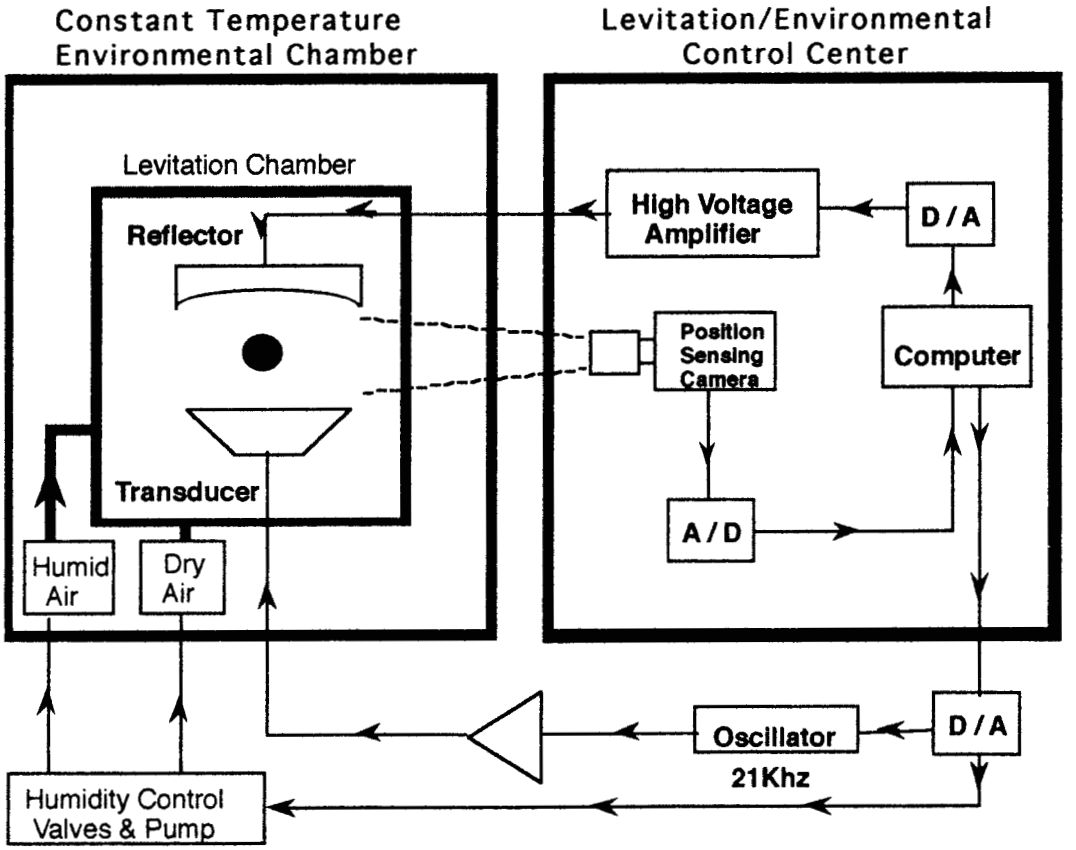

FIGURE 1. Schematic description of the ultrasonic electrostatic hybrid system.

period of time.

In order to apply the ultrasonically induced rotation on suspended drop, a small translational adjustments are made either to the reflector or the transducer with respect to their cylindrically symmetrical position. The origin of this torque was hypothesized to be directly linked to the steady acoustic streaming flows induced by the high intensity sound waves (Trinh and Robey, 1994). Measurements of acoustic streaming flow around the rotating sample has revealed this to be true. Figure 2 is a recent result of PIV(particle image velocimetry) performed through the center of droplet orthogonal to the rotion axis. The imaging of flow field is made by a thin laser sheet and a CCD video camera taking images of scattered light from suspended smoke particles. A direct correlation appears evident between the air flow vortices and the sample rotation direction. The rotation rate for a suspended sample depends primarily on its size, shape and on the acoustic field parameters (frequency and amplitude). The orientation of the rotation axis about X-Y

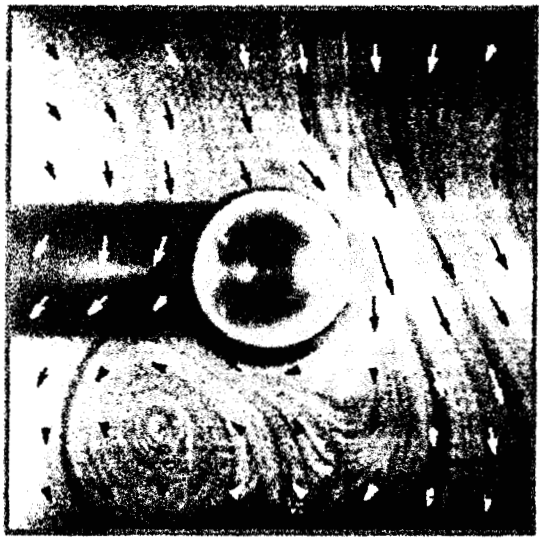

FIGURE 2. Video frame showing the patterns of acoustic streaming flows past a levitated drop in the rotating states. Arrows indicate the relative magnitude and the direction of the flow obtained from 2-D PIV.

the plane(the plane parallel to the face of the transducer) can be made by arranging the position of reflector or transducer as described in figure 3. A small, limited range of tilt control of the rotation axis is also possible by adjusting the acoustic frequency. The rotation rate for a protein solution is varied, typically, between 1 and 3 revolutions per second during nucleation and growth experiments.

The overall behavior of the droplet , the internal flows within the droplet and the crystals within the droplet are monitored by two video cameras equipped with long-distance microscopic lens. The drop volume is monitored by using digitally captured drop contours (assuming the drop is axisymmetric) (Chung et al, 1996) or the changes in the high voltage value required for levitation (assuming the surface charge is held constant).

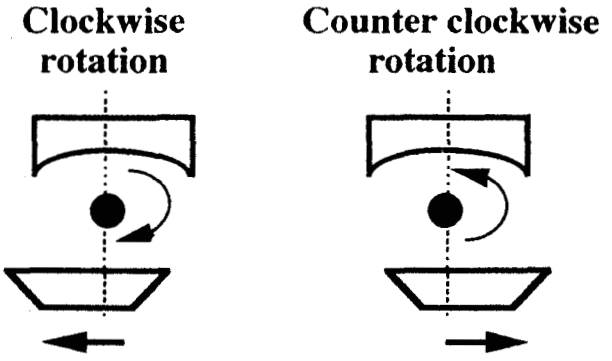

FIGURE 3. Rotation orientation can be controlled by moving the transducer position with respect to the equilibrium position

2. PARTICLE MOTION WITHIN A ROTATING LEVITATED DROP IN 1G

The ideal simulation of Microgravity environment in a protein solution droplet would mean conditions that exclude sedimentation, clumping(twinning) of crystals and formation of crystals at the solution-gas interface. The condition would require the creation of an isotropic distribution of all the relevant physical parameters within a region centered on a developing crystal nucleus (McPherson, 1996)(Malkin et al, 1995)(Lin et al, 1995)(Rosenberger et al, 1996)(Long et al, 1996). The objective of this project is thus to be able to nucleate and to keep the crystals within the bulk of the levitated solution by averaging out the effective acceleration felt by the crystal through the drop rotation along a horizontal axis. The drop rotation speed should be fast enough such that the time constants required for diffusive and residual convective motions of fluid that could affect the distribution of concentration near the crystals are much longer compared to the period of rotation.

Since the levitated protein solution drop is charged(to about $4x10^{-10}$ Coulomb), an observation was made to detect a possible internal flow that may be induced by the surface charge. Following of tracer particles that closely matches the density of solution suspended within the droplets has shown the absence of any substantial flow once the humidity and the thermal equilibrium is reached. This ruled out the existence of any internal flow induced by the surface charge distribution and the electric field. Theoretically, since the drop is nearly spherical and essentially a conductor carrying a surface charge, the electric field inside should be close to zero.

The initial experimental study of the internal flows in an electrostatically suspended rotating drop in the transient and steady-state phases has revealed more complicated motion than first anticipated. A slow lateral motion(order of 0.5mm/min) riding on top of circular motion of the rotation of the drop is observed. The lateral motion circulated through the rotation axis, then out towards the surface making its way back into the bulk solution from the other side of the rotation axis and repeating. This peculiar circulation was originally thought to be brought about due to the density difference between the tracer particle and the solution combined with slightly tilted rotation axis. A recent observation, however, ruled out that theory after

observing the flow pattern produced with two different combination of density of the tracer particles and the solution. Since the surface charge does not seem to play an important role for the internal flow, the only plausible explanation is the effect of acoustic streaming flow to the surface of the drop. Experimentally, it was found that the lateral migration of the fluid can be significantly varied depending on the multiple physical parameters such as the driving frequency, and mechanical alignments of reflector and the transducer. A three dimensional PIV experiment is being devised to more carefully study the momentum transfer of acoustic streaming flow to the surface of the drop that ultimately seem to produce a dominating internal flow. One additional factor that produces the lateral motion is the transient flows induced in the droplet by changes in the rotation velocity. The effectiveness of transient flows induced during the spin-up phase of the rotating liquid-filled container has already been observed previously (Annamalai, 1983). All observations of internal flows are carried out at constant rotation rate by using a rotation control scheme described earlier.

3. PROTEIN CRYSTAL GROWTH IN LEVITATED ROTATING DROPLETS:PRELIMINARY STUDY

The levitation-rotation approach was tested by using Hen Egg White Lysozyme (Sigma Chemical Co.) solutions at 3.5% (wt) lysozyme, 4% NaCl in 0.02M acetate buffer solution at

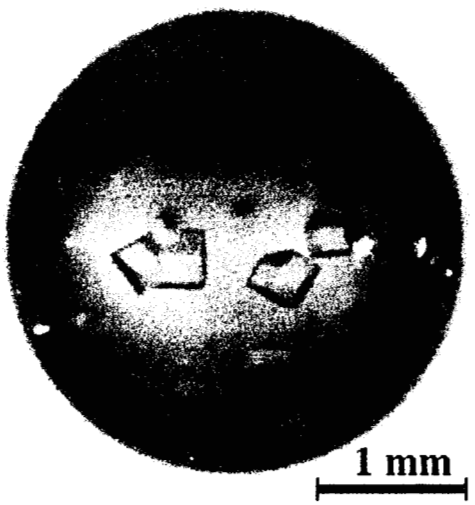

FIGURE 4. Lysozyme crystals grown from a non rotating levitated solution droplet.

pH 4.3. Typically, 17 μl of solution was used to launch a droplet into the levitator. Initially, crystal growth was attempted with stationary, non-rotating levitated droplets at 20 degrees C. The result is presented in figure 4. Most crystal were found clustered near the inner boundary of drop-air interface with the majority at the bottom. A few crystals were found suspended near the upper pole on the inside of the droplet surface. Isolated crystals were also found on the side wall of the droplet, suggesting mutual repulsion through induced or residual surface charges. None of the crystals actually broke the surface of the droplet as the solution totally wetted all their facets.

Crystals of Lysozyme were grown in pure solutions under horizontal rotation with rate between 1 and 3 rps. Initially, small crystallites(about 5μm) formed in the droplet bulk were orbiting about the rotation axis without sedimenting to the bottom or migrating to the outer surface, a clear indication that in spite of the substantial density difference between the crystals and the solution, suspension could be maintained through rotation. As the crystals grew larger, however, the net force acting on the crystal changed as the size and the mass of the crystals evolved that a constant rotation rate was not adequate to hold the crystals in the bulk. A quick control of rotation rate to maintain a fine balance of drag and centrifugal force would be necessary to hold the crystals in the bulk for extended period of time. When the crystallites were small(< 5μm), complicated trajectories accompanied by slow lateral motion parallel to the axis of rotation were also observed as in the case of the study performed with tracer particles. Due to the greater density of Lysozyme crystals, the recirculation of the crystallites were not as readily encountered as the case of the tracer particles, and most of the crystallites that migrated toward one side ended up being stuck near the pole of the rotation axis.

In order to reduce the affects of lateral fluid motion caused by the external acoustic streaming flow and at the same time, increase the drag force to counter the centrifugal force, we have tried additives to the protein solutions. A minute addition of agarose gel into the protein solution was used for crystal growth experiments using levitated rotating droplets. The reason for the addition of agarose gel over other agents is its well known bio-compatibility to the protein solution and the numerous experimental data observed by other authors (Robert and Lefaucheux, 1988)(Provost and Robert, 1991). The normal concentration of agarose is 0.1% (wt) in order to achieve a gel state and completely eliminate convective contributions. The amount of agarose used in our study was on the order of 0.01%, and the mixture displayed liquid-like properties with slower sedimentation of suspended crystals in a non-rotating droplet. With the initial concentration of 0.006 wt% agarose, we were able to maintain Lysozyme crystals in suspension in the droplet bulk under rotation . Crystals were grown to a size of about 300 μm. Upon stopping rotation the crystal would very slowly sediment to the bottom of the droplet, but could be made to settle within seconds by inducing violent shape oscillations of the droplet. Figure 5 shows lysozyme crystals growing in a horizontally rotating droplet viewed at two extreme rotation angle. Under equilibrium conditions where the temperature and the rotation rate are held constant, video tracking indicates no detectable differential motion of the crystals over a prolonged period. Any sudden change in rotation velocity (angular acceleration), however, still produced lateral motion parallel to the rotation axis as it was the case with pure solution.

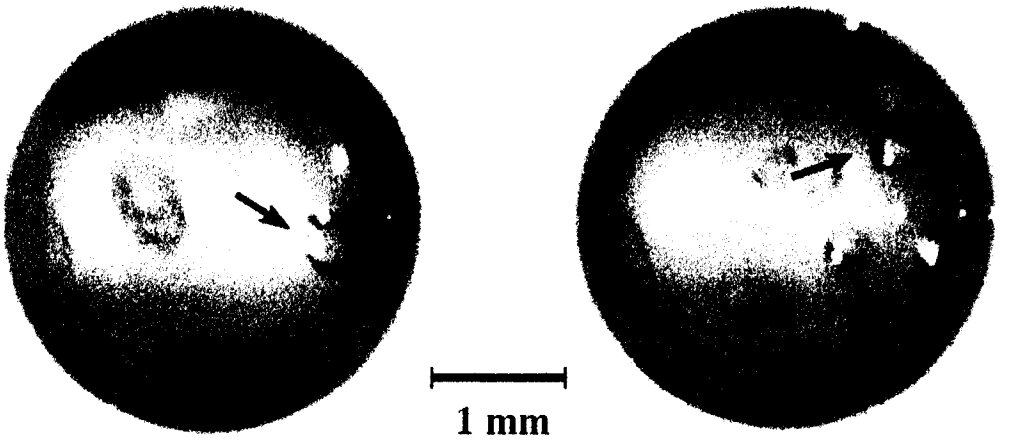

FIGURE 5. Lysozyme crystals grown from a rotating levitated solution droplet with 0.01wt% agarose additive.

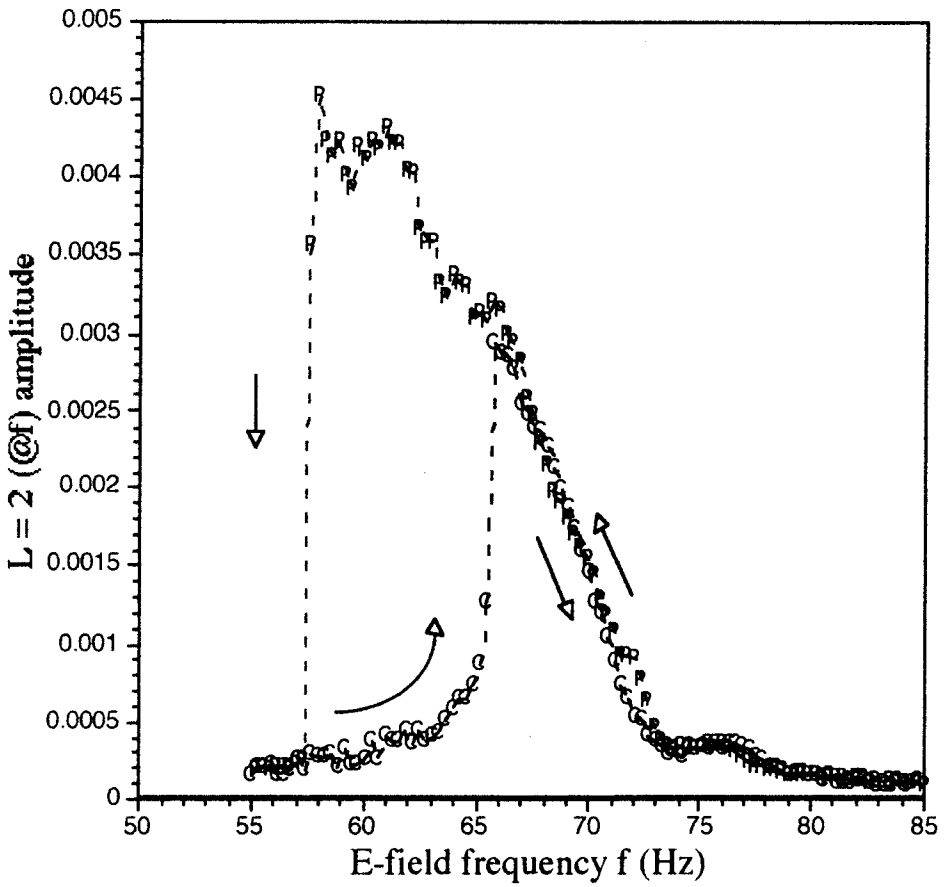

FIGURE 6. Hysteresis in large amplitude shape oscillations of an ultrasonically levitated drop driven by a time-varying electric field. Much larger amplitude are achieved during the downwards frequency sweep than during the upward sweep. The lowest frequency attained prior to a sudden decrease in amplitude is over 10 Hz lower than the resonance frequency located during the upward sweep.

4. LARGE-AMPLITUDE ELECTRICALLY-DRIVEN SHAPE OSCILLATIONS OF ULTRASONICALLY LEVITATED UNCHARGED DROPS

A sub-system of the apparatus designed for containerless crystal growth can be used for the study of nonlinear drop dynamics in 1 G. In this particular instance, the reflector of the ultrasonic levitator was the high voltage electrode, and it was attached to the source of a low frequency sinusoidal signal used to induce shape oscillations in an uncharged water drop levitated in air. By varying the frequency of the time-varying electric field, the successive shape oscillations normal modes were excited. The drops were about 3 mm in diameter, and had an oblate equilibrium shape due to the high ultrasonic pressure required for levitation. Residual dissolved salts in the water allowed the periodic polarization and deformation of the drop surface in the time-varying electric field. This resulted in a periodic elongation of the drop along the vertical axis, the restoring force being provided by surface tension. The drop motion was recorded by a high-speed video camera operating at up to 2,000 frames per seconds, and the digitized images were analyzed in order to obtain time series as well as the Fourier spectra of the oscillations.

Sub-harmonic excitation of the resonant modes were observed where resonant mode oscillations were driven at half the electric field frequency ω_e (together with a response at ω_e). These results have already been reported elsewhere (Trinh et al, 1995) and they will not be discussed here. In this paper we report additional observation relating to the hysteresis observed with high amplitude driven oscillations. As shown in figure 6, a significant hysteresis is observed when the electric field frequency is swept down past the observed resonance peak found during the upward frequency sweep. Increasingly larger oscillation amplitude can be induced at significantly lower frequency until a sharp decrease is obtained. The detailed observation of the oscillations in the vicinity of this sharp drop has revealed the onset of a shape instability prior to the loss of oscillations. As shown in figure 7 where images of the drop shapes at various phases of the oscillation cycle are shown both prior and during the onset of the instability. At the instability onset, the initially pure oblate-prolate vibration are replaced by a more complex motion resulting from a superposition of a lateral wobbling at a different frequency. Soon after the first appearance of this additional motion, most of the energy is suddenly lost to the shape oscillations. A similar hysteresis has been observed for drop oscillating within a *liquid* medium, but the decrease in amplitude at the lowest frequency was not nearly as sudden as observed for a drop in air.

5. SUMMARY

The feasibility of growing Lysozyme crystals from solution in levitated and horizontally rotating droplets has been demonstrated by utilizing the capabilities of an ultrasonic-electrostatic hybrid levitation system. Preliminary data on the long term suspension behavior of macroscopic crystals have been obtained. They suggest the refinement of experimental control of the rotational velocity and the closer understanding of the lateral fluid motion along the rotation axis. The use of solution additives such as agarose has yielded some promising results, and has provided some experimental input for the fluid dynamic modeling of the crystal suspension-rotating solution droplet problem. Future studies are focused on the analysis and experimental characterization of the flow environment of the growing crystals in order to assess the feasibility of simulating low-gravity conditions in 1 G.

The combination of the electric and ultrasonic allows a new set of experiments to be carried out in order to probe the details of the nonlinear motion of free drops. This enhanced flexibility in experimentally adjusting the equilibrium shape, the strengths of both the electric and ultrasonic fields, the free charges carried by the drop, and the relative strength of electric and acoustic radiation stresses should permit a more rigorous assessment of the field effects on the drop dynamics, and should lead to a more rigorous evaluation of the benefits of a low gravity environment for the study of free drops.

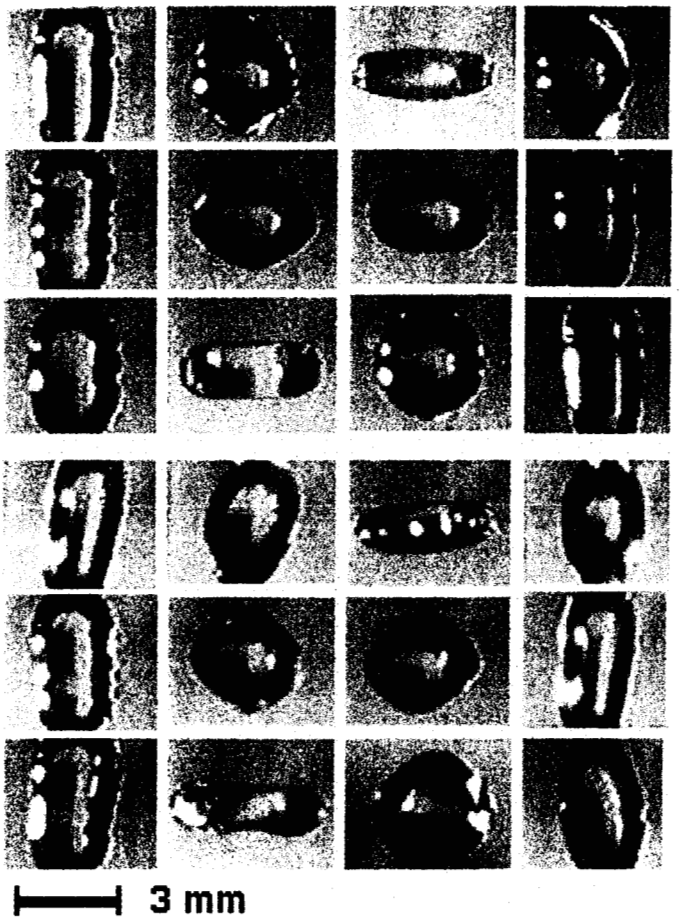

FIGURE 7. Onset of lateral instability at large amplitude shape oscillations(60 Hz). The upper set of photographs describe stable large amplitude n=2 axisymmetric oscillations. The lower set shows the wobbling motion superposed upon the oblate-prolate shape oscillations precursor of the sudden decrease in oscillation amplitude.

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